1/24/00

UNITED STATES DISTRICT COURT

DISTRICT OF MASSACHUSETTS

AMGEN INC., Plaintiff,	)	Civil Action No. 97-10814-WGY
v.	)	
HOECHST MARION ROUSSEL, INC. and TRANSKARYOTIC THERAPIES, INC., Defendants.	)	

EXPERT REPORT OF

RICHARD D. KOLODNER, PH.D.

I. BACKGROUND AND QUALIFICATIONS

1. I am a Professor of Medicine, Member of the Cellular and Molecular Medicine Program at the University of California, San Diego School of Medicine, and Head of the UCSD Cancer Center/Cancer Genetics Program. I am a Member of the Ludwig Institute for Cancer Research and Head of the Laboratory of Cancer Genetics at the Ludwig Institute San Diego Branch. For the past 29 years, I have conducted research in the fields of molecular biology and biochemistry. I received a B.S. and Ph.D. in Biological Sciences from the University of California, Irvine, and was a postdoctoral fellow at Harvard Medical School from 1975-1978. From 1978 to 1997, I was a Professor at Harvard Medical School in the Department of

Biological Chemistry and Molecular Pharmacology and the Dana-Farber Cancer Institute Department of Cancer Biology.

2. For the past 22 years, I have studied the mechanisms of homologous recombination and DNA repair, encompassing studies in bacteria, yeast, and mammalian cells. In 1983-1984, I was actively engaged in studies of homologous recombination in bacteria and yeast. In 1996, I received the Charles S. Mott Prize for my work involving fundamental studies on DNA mismatch repair and the relationship between defects in DNA mismatch repair and cancer susceptibility. DNA mismatch repair plays an important role in both homologous recombination and DNA replication. I have been an associate editor of the journals *Cell* and *Cancer Research* since 1995 and 1996, respectively. Since 1999, I have served on the editorial board of the journal *Molecular and Cellular Biology*. I have co-authored more than 150 scientific articles, including numerous articles regarding homologous recombination. Attached as Exhibit A is a copy of my *curriculum vitae*.

3. During this litigation, I prepared and submitted a declaration dated December 7, 1999, in support of Amgen's opposition to defendants' motion for summary judgment of invalidity, which is attached as Exhibit B. As the statements and opinions made in my December 7, 1999 declaration are relevant to the testimony that I intend to give at trial, I hereby incorporate by reference the entirety of that declaration into this report.

4. I submit this report in support of Amgen's positions on the validity of the claims at issue of U.S. Patent Nos. 5,547,933, 5,618,698, 5,621,080, 5,756,349, and 5,955,422 (individually referred to by the last three digits only; collectively referred to as "Dr. Lin's patents").

II. MATERIALS CONSIDERED

5. In forming the opinions expressed here, I have considered the following:

- U.S. Patent No. 5,547,933 (the "'933 patent") (I have been informed that the specification of this patent is the same as that of the other four patents involved in this litigation);

- The claims of U.S. Patent Nos. 5,621,080 (the "'080 patent"); 5,955,422 (the "'422 patent"); 5,547,933 (the "'933 patent"); 5,756,349 (the "'349 patent"); and 5,618,698 (the "'698 patent").

- The December 20, 1999 expert report of Dr. Robert E. Kingston and its supporting references.

- Defendants' Contingent Cross-Motion for Summary Judgment of Validity, dated November 23, 1999.

- The references attached as Exhibit C.

- Portions of defendant HMR's Investigational New Drug Application,

6. Additionally, in forming my opinions, I have relied upon the knowledge, training, and experience that I have acquired from my more than 29 years of research in molecular biology, biochemistry, and human genetics.

7. Throughout Dr. Kingston's report, including paragraphs 7, 26, and 63, he alludes to numerous topics on which he may elect to provide an opinion at a future date. This description of Dr. Kingston's intended testimony, however, is too general and vague to afford me a reasonable opportunity to formulate a response. If the Court allows Dr. Kingston to introduce additional statements or opinions at trial, I reserve the right to supplement my opinions accordingly. To the extent that his report provides specific examples and opinions, these have been addressed in this report.

8. I further reserve the right to supplement or amend my opinions in light of any additional evidence, testimony, or other information, including any claim construction that the Court may enter, and that may be provided to me after the date of this report. Also, in the event that defendants' witnesses are permitted to raise issues at trial not present in their expert reports, I may also testify about these additional issues.

III. GENE TARGETING VIA HOMOLOGOUS RECOMBINATION

9. The following is a brief tutorial that I intend to provide at trial, to assist the Court with issues relating to "gene targeting" via "homologous recombination." In my tutorial, I will introduce key terms and describe the tools of "gene targeting" via "homologous recombination."[1]

A. Basics of Gene Targeting Via Homologous Recombination

10. "Gene targeting" is a genetic manipulation using recombinant DNA molecules and the introduction of "exogenous" DNA into a cell, ultimately resulting in an altered, non-natural recombinant gene in a chromosome, into which the "exogenous" DNA has integrated. Gene targeting was originally developed to alter natural chromosomal genes or sites for producing mutant cell lines in animals and for gene therapy useful in treating human genetic disorders.

[1] Attached are Exhibits C1, C2 and C3, three review articles summarizing basic background on "gene targeting" and "homologous recombination": M. Capecchi, *Scientific American* (March 1994), M. Capecchi, *Trends in Genetics*, 5(3):70-76 (1989); M. Capecchi, *Science* 244:1288-1292 (June 1989). Also attached are book chapters providing background details on the properties and underlying mechanisms of homologous recombination, on which I may rely. *See* Anthony J.F. Griffiths, et al., AN INTRODUCTION TO GENETIC ANALYSIS, 6th ed. (W.H. Freeman and Co., New York, New York, 1996) Chapter 20, *Mechanisms of Genetic Change II: Recombination*, pp. 617-636 (Exhibit C4) and MOLECULAR CELL BIOLOGY (J. Darnell et al., eds.) (Scientific American Books distributed by W.H. Freeman and Co., New York, New York, 1986), pp. 555-565 (Exhibit C5).

11. Defendants use gene targeting merely as a tool for site-specifically altering the DNA sequence of a cell's chromosome by inserting a DNA sequence into the cell at a desired location (the "target" gene or site). The end result of this use of gene targeting via homologous recombination is to replace all or part of a "native" gene or site (the "target") in the chromosome with a modified gene or DNA sequence (introduced from outside the cell), thereby creating a recombinant DNA chromosome with a gene whose structure (and possibly function) have been altered from its natural state. Thus, like PCR (polymerase chain reaction) and automated DNA synthesis, defendants' use of gene targeting via homologous recombination is simply an additional tool for manipulating DNA to create recombinant DNA molecules and recombinant cells useful in producing recombinant proteins.

12. Generally speaking, homologous recombination can be used to make two DNA molecules, having identical or nearly identical sequences, recombine to form two new hybrid molecules. The key requirement for this process is that the two stretches of DNA have sequences that are "homologous," i.e. sequences that are identical or nearly identical and of sufficient length to permit recombination.[2] If two DNA strands are not sufficiently identical, they will not recombine. If two DNA strands share identical sequences that are not of sufficient length, they will not recombine. Homologous recombination tools involve the exchange of a DNA sequence from one DNA strand to another, resulting in formation of two new recombinant DNA molecules.

13. When DNA molecules have been acted upon by homologous recombination, they appear to have participated in the following steps:

(a) sequence pattern matching and alignment — the homologous DNA molecules first line up (i.e. pair at regions of homology) with each other,

[2] *See* Rubnitz and Subramani, *Mol. Cell. Biol.* 4:2253-58 (1984) (Exhibit C6).

(b) cleavage — during recombination, the DNA molecules behave as if they had been cut at identical sequences within each recombining DNA, and

(c) joining — after recombination, the apparently cleaved DNA molecules appear to have been joined to each other at their cleaved ends with such precision that sequences at the points of linkage are not altered.

14. Homologous recombination techniques can be used to recombine two chromosomal DNA molecules or two non-chromosomal DNA molecules having identical or nearly identical sequences upon introduction into a mammalian cell. These basic techniques are used in gene targeting to alter a chromosomal DNA sequence within a cell with a non-chromosomal DNA sequence that is introduced into the cell.

15. Gene targeting requires knowledge of sufficient DNA sequence of the gene to be targeted and/or the surrounding DNA. Without such knowledge, one cannot design or select the targeting vector so that it will recombine into the desired chromosomal locus.

16. A "targeting vector" is a vehicle for delivering a desired DNA insertion or alteration into a "target gene" in a cell's chromosomal DNA. An example of a "targeting vector"[3] is a recombinant DNA molecule that contains: (a) a DNA sequence that is sought to be introduced into the chromosome to alter the natural target gene; (b) two "flanking sequences" that flank the DNA sequence modification that will be introduced into the cell to alter the natural target gene, where such "flanking sequences" have a sufficient length of sequence identity with the target gene to permit homologous recombination to occur within each flanking region. In this example, the occurrence of two recombination events, one within each flanking sequence, results in replacement of the original chromosomal DNA between the two recombination sites with the modified DNA sequence that was in the "targeting vector" introduced into the cell at the desired location.

[3] Also called a "targeting construct."

17. The "targeting" of the "exogenous" DNA into a particular gene in a chromosome requires that the "exogenous" targeting vector contain DNA sequences that are sufficiently "homologous" to the target gene. Thus, in order to achieve gene targeting, one must first have the DNA sequence of a cloned DNA from the chromosomal DNA "target gene" that is to be altered. Without knowledge of the "target gene" DNA sequence or similarly detailed structural information, one cannot use the homologous recombination technique to alter specifically a chromosomal target gene inside a cell. For example, if one wished to alter the human insulin gene, one would first need to obtain a DNA sequence of sufficient length that is within or near the human insulin gene.

18. A 1994 *Scientific American* article[4] described gene targeting in cells:

> "The first step is to clone the gene of interest and propagate it in bacteria. This procedure provides a pure source of DNA containing the gene. . . . Next, in a test tube, the nucleotide sequence of the gene is changed to meet the purpose of the experiment. The altered gene is referred to as the targeting vector. The targeting vector is introduced into living cells by any of several means. Once within the cell nucleus...it searches through all the sequences of the genome until it finds its counterpart (the target). If it indeed does find its target, it will line up next to that gene and replace it."

19. The foregoing makes clear that gene targeting using homologous recombination requires, at a minimum: (1) knowledge of or possession of a piece of DNA having the identical sequence of the chromosomal target gene that is to be altered from its native state; (2) design of a recombinant, "exogenous" targeting vector to be introduced into a cell to replace a "target" gene or DNA sequence; (3) introduction of that "exogenous" targeting vector, having identical or nearly identical sequences of sufficient length to the "target"; and (4) knowledge of sufficient chromosomal DNA sequence and gene structure of the target gene to determine how one wishes to alter it. Thus, gene targeting via homologous recombination techniques alone, without the

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[4] *See* Exhibit C1, M. Capecchi, *Scientific American*, p. 56 (Mar. 1994).

sequence or structure of the "target gene," is useless. That is why defendants could not have achieved gene targeting to express EPO had they not had the benefit of Dr. Lin's teachings of, among other things, the Figure 6 DNA sequence to design their targeting vector.

20. Since the "target gene" is altered from its native state after gene targeting, it is no longer a natural gene but rather an artificial, altered recombinant gene containing "exogenous" DNA.

21. Also, any protein produced using gene targeting via homologous recombination is made using recombinant DNA (the altered target gene) and "exogenous" DNA expression and therefore, would be a recombinant protein produced by "exogenous" DNA expression.

B. Development of Gene Targeting Via Homologous Recombination

22. With the advent of recombinant DNA techniques, molecular biologists were interested in the use of isolated DNA molecules to alter the chromosomes of mammalian cells. Homologous recombination experiments using recombinant DNA technology were first conducted in bacteria and yeast in the 1970's and paralleled the development of the field of molecular biology. Studies of targeted homologous recombination in mammalian somatic (non-egg or sperm) cells began with examination of non-chromosomal homologous recombination events observed in plasmid DNA molecules following their introduction into mammalian cells.[5]

23. These initial experiments led the way for homologous recombination studies using chromosomal target sequences containing integrated plasmid molecules.[6] For example, in 1985, Lin et al. described homologous recombination in mammalian cells. There, a defective *tk* gene, missing a portion of its 5′ end, was introduced into mouse cells and was integrated at a random chromosomal site. Then, homologous recombination was used to target a *tk* gene

[5] Folger et al., *Mol. Cell. Biol.*, 2(11):1372-1387 (Nov. 1982) (Exhibit C7).
[6] *See* F.-L. Lin et al., *PNAS USA* 82:1391-1395 (Mar. 1985) (Exhibit C8).

missing a portion of its 3′ end, but containing an intact 5′ region, into the same mouse cells, thus restoring a functional *tk* gene In the mid-1980's, researchers demonstrated targeted plasmid integration into the human β-globin locus in cultured mammalian cells.[7]

IV. SUMMARY OF OPINIONS

Opinion 1

In 1983, an ordinarily skilled molecular biologist would not have considered Dr. Lin's teachings and inventions to be limited to uses of "exogenous, cloned" EPO DNA.

Opinion 2

At the time of Dr. Lin's inventions, an ordinarily skilled molecular biologist would have understood Dr. Lin's patents to describe the expression of DNA encoding human EPO in mammalian cells to achieve high-level production of EPO.

Opinion 3

At the time of Dr. Lin's inventions, an ordinarily skilled molecular biologist would have understood Dr. Lin's patents to teach expression of DNA encoding human EPO in mammalian cells to achieve high-level production of EPO using only routine experimentation.

Opinion 4

One of ordinary skill in the art would not have accepted Dr. Kingston's various interpretations of Dr. Lin's specification.

Opinion 5

Defendants' genetic manipulation of mammalian cells to produce EPO is neither "pioneering" nor "third-wave."

Opinion 6

Defendants could not have achieved expression of human EPO in human cells without copying Dr. Lin's inventions.

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[7] *See* O. Smithies et al., *Nature* 317:230-234 (1985) (Exhibit C9).

V. BASES AND REASONS FOR OPINIONS

Opinion 1

In 1983, an ordinarily skilled molecular biologist would not have considered Dr. Lin's teachings and inventions to be limited to uses of "exogenous, cloned" EPO DNA.

24. Dr. Kingston asserts that one of ordinary skill in the art, in 1984, would have understood Dr. Lin's patents to be limited to the uses of an exogenous, cloned EPO DNA.[8] I disagree. Dr. Kingston premises his assertion upon a distinction between "cloned, exogenous," and "endogenous." One of ordinary skill in the art, however, would have found this distinction to be scientifically meaningless.

25. Dr. Kingston attempts to create an artificial distinction between what he calls a "cloned exogenous" EPO DNA and an "endogenous" or "native" EPO DNA. The terms "cloned," "exogenous" and "endogenous" are merely labels that Dr. Kingston uses to describe the same DNA sequence, *i.e.*, the natural human EPO gene. Dr. Kingston's focus on the location of the DNA sequence, *i.e.*, whether it is a "cloned" piece of DNA or whether it is residing in the chromosome, is a distinction without scientific significance because the true utility of the information contained in any piece of DNA is not its location, but its sequence and organizational structure.

26. Before Dr. Lin's breakthrough inventions, the world had no access to the human EPO gene. Elucidating its sequence and structure, Dr. Lin gave the world the ability to use the human EPO gene, whether inside or outside of a cell. An ordinarily skilled molecular biologist would have understood that Dr. Lin's Figure 6 DNA sequence is the sequence of the "endogenous" human EPO gene that naturally resides in chromosome 7 of human cells. When Dr. Lin "cloned" the human EPO gene, he isolated it from a collection (or "library") of DNA

sequences, all from the natural chromosomes of a human cell. One of the many advantages of having the cloned human EPO gene is that it permits characterizing its structure and function. But, that DNA sequence is still the same DNA sequence that exists naturally within human cells.

27. Because the detailed structural information provided by Dr. Lin describes both the "endogenous" and "exogenous" human EPO gene, the distinction in terms of its location is scientifically unimportant. The true significance of what Dr. Lin achieved by cloning the human EPO gene is that he provided an accurate description of its sequence and structure, which made possible, for the first time, the production of therapeutic EPO, cells capable of producing therapeutic EPO, and numerous other materials and methods involving the human EPO protein and gene. The structure and sequence of the human EPO gene and the protein it encodes are essential keys to using the EPO gene in novel ways regardless of its location inside or outside of the cell.

28. Indeed, only by appropriating Dr. Lin's seminal teachings were defendants able to use what Dr. Kingston labels as the "endogenous" human EPO gene. For example, defendants could not have targeted an "exogenous" viral promoter and an "exogenous" DHFR gene to the EPO gene without their knowledge of the DNA sequence of the EPO gene and use of sequences that hybridize under stringent conditions to the Figure 6 sequence reported in the patents. As explained in detail below, the homologous recombination technique that defendants used to insert their viral promoter and DHFR gene into the human EPO gene requires the presence in their targeting construct of sequences that are identical or nearly identical to the EPO gene.[9]

[8] Kingston ¶ 28.
[9] Kolodner Decl. ¶¶ 7, 9.

29. When one clones a DNA containing a gene, one does not envision using that gene in only its cloned form. For example, an ordinarily skilled molecular biologist studying human genetics would have contemplated using the gene to study allelic variants or disease-causing mutations within cells from many individuals. The structure of the gene and the protein it encodes permits a vast number of applications, including diagnostics, transgenic animals, mapping the gene and other nearby genes, and cloning regions of DNA linked to the EPO gene, just to name a few described by Dr. Lin.

30. An ordinarily skilled molecular biologist would have considered many applications of the inventions disclosed in Dr. Lin's patents not limited to mere uses of the cloned version of the gene. For example, manufacturing synthetic EPO polypeptides[10] does not require use of an "exogenous," cloned DNA sequence, but rather only knowledge of the amino acid sequence of the desired protein or polypeptide. Preparation of monoclonal antibodies to EPO polypeptides or to recombinant human EPO[11] also does not require use of the cloned DNA sequence, only use of recombinant or synthetic human EPO or fragments thereof. Synthetic DNA sequences encoding human EPO[12] can be prepared based on the amino acid sequence of human EPO, which before Dr. Lin's work was never possessed by any researchers. Similarly, a molecular biologist would have understood that the pharmaceutical human EPO purified from mammalian cells in culture, methods of producing human EPO, and vertebrate cells capable of producing human EPO in therapeutically useful quantities are not limited to cells having an "exogenous" EPO DNA sequence.

31. Dr. Lin's patents contemplated use of DNA sequences more broadly than just "exogenous" cloned EPO DNA; it also contemplated uses of "endogenous" EPO DNA:

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[10] *See* '933 Col. 34:42-67.
[11] *See id.*

> "Novel DNA sequences of the invention include ***all sequences useful in securing expression in procaryotic or eucaryotic host cells*** of polypeptide products having at least a part of the primary structural conformation and one or more of the biological properties of erythropoietin which are comprehended by: (a) the DNA sequences set out in FIGS. 5 and 6 herein or their complementary strands; . . . Specifically comprehended in part (b) are genomic DNA sequences encoding allelic variant forms of monkey and human erythropoietin. . ."[13]

Dr. Kingston, however, attempts to read "cloned" or "exogenous" into the phrase "all sequences useful in securing expression..."[14] An ordinarily skilled molecular biologist at the time of invention would have understood this passage to make plain that the inventions were not limited to "cloned" versions, but rather to the "sequences," regardless of whether they are in "cloned" form or located in their native chromosomal locus. For example, one of skill would have considered "endogenous" the multiple sequences of the human EPO gene within the cells of Example 10 of Dr. Lin's specification to the extent that such sequences came into existence within the cells and are identical to the sequence of the "endogenous" human EPO gene. Thus, one of skill would have understood such non-exogenous sequences to exemplify novel DNA sequences of the invention useful in securing expression.

32. Another relevant passage makes plain that Dr. Lin's inventions could be used to identify upstream DNA sequences within a human or other mammalian chromosome and to identify allelic variants:

> "DNA products of the invention may also . . . [be] employed in DNA hybridization processes to ***locate the erythropoietin gene position and/or the position of any related gene family in the human, monkey and other mammalian species chromosomal map. They can also be used for identifying the erythropoietin gene disorders at the DNA level and used as gene markers for identifying neighboring genes and their disorders.***"[15]

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[12] *See* '933 Col. 29-32.
[13] '933 Col. 11:41-6.
[14] Kingston ¶ 48.
[15] '933 Col. 12:12-21.

33. An ordinarily skilled molecular biologist therefore would have considered Dr. Lin's inventions to include all uses of the information in the patent relating to sequence and structure of the human EPO gene, and would not have limited his inventions just to the uses of an exogenous, cloned piece of EPO DNA.

Opinion 2

At the time of Dr. Lin's inventions, an ordinarily skilled molecular biologist would have understood Dr. Lin's patents to describe the expression of DNA encoding human EPO in mammalian cells to achieve high-level production of EPO.

34. Dr. Kingston asserts that as of 1984 one of ordinary skill would not have understood Dr. Lin to have described or possessed any inventions that included expression of EPO in a human cell from an endogenous EPO DNA.[16] He premises this assertion on the ground that Dr. Lin's claims all require exogenous EPO DNA.[17] One of ordinary skill in the art, however, would have understood that the claims at issue are not limited to the manner in which the recited cells were genetically engineered, and specifically are not limited to the use of EPO DNA in a particular location in the vertebrate cells to make human EPO.[18] As shown below, one of skill would have concluded that Dr. Lin's patents describe the vertebrate cells as claimed.

35. For example, claim 1 of the '349 patent specifies:

> "Vertebrate cells which can be propagated in vitro and which are capable upon growth in culture of producing erythropoietin in the medium of their growth in excess of 100 U of erythropoietin per 10^6 cells in 48 hours . . . said cells comprising non-human DNA sequences which control transcription of DNA encoding human erythropoietin."

[16] Kingston ¶ 52.
[17] Kingston ¶ 48.
[18] Additionally, reading the claims at issue in the context of the patents' other claims, one of skill would have noted that none of the claims at issue contain any limitation to a "cloned" or "exogenous" EPO DNA sequence, whereas other claims not at issue do specify "exogenous" DNA. (*See, e.g.*, '933 claim 6.) One of ordinary skill in the art therefore would have found inappropriate Dr. Kingston's attempt to limit the claimed inventions to only those embodiments possessing an "exogenous" EPO DNA sequence.

From this language, one skilled in the art would have understood that the claimed vertebrate cells, including human cells, must: (1) be able to produce the specified amount of EPO in their culture medium, and (2) have non-human transcription control sequences which control transcription of EPO DNA.

36. As the following passages of the specification reflect, one of ordinary skill would have understood Dr. Lin to have described vertebrate cells possessing the required characteristics:

> "***Vertebrate*** (e.g., COS-1 and CHO) ***cells*** provided by the present invention comprise the first cells ever available which can be ***propagated in vitro continuously and which upon growth in culture are capable of producing in the medium of their growth in excess of 100 U*** (preferably in excess of 500 U and most preferably in excess of 1,000 to 5,000 U) ***of erythropoietin per 10^6 cells in 48 hours as determined by radioimmunoassay.***"[19]

> "Collectively referred to as "promoter/regulator" or ***"control" DNA sequence***, these sequences which precede a selected gene (or series of genes) in a functional DNA polymer cooperate to ***determine whether the transcription*** (and eventual expression) ***of a gene will occur.***"[20]

> ***"FIGS. 6A, 6B, 6C, 6D, and 6E (collectively referred to as Fig. 6) show the sequence of*** human genomic EPO DNA ***and the*** encoded EPO."[21]

> "In the following construction, the ***EPO gene*** is altered so that it is ***expressed using the SV40 late promoter.***"[22]

37. Given the extensive description in the specification, one of ordinary skill would have concluded that Dr. Lin had fully described and was in possession of the claimed cells at issue.

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[19] '933 Col. 10: 41-49 (emphasis added).
[20] '933 Col. 2: 8-13.
[21] '933 Col. 13: 28-30.
[22] '933 Col. 24: 12-14 (emphasis added).

Opinion 3

At the time of Dr. Lin's inventions, an ordinarily skilled molecular biologist would have understood Dr. Lin's patents to teach expression of DNA encoding human EPO in mammalian cells to achieve high-level production of EPO using only routine experimentation.

38. An ordinarily skilled molecular biologist would have understood Dr. Lin's patents to give detailed and comprehensive guidance regarding the production of human EPO in mammalian cells in culture, which could have been followed and reproduced in many different ways with only routine experimentation. Dr. Lin's patents describe a tremendous amount of inventive research, including the first available therapeutic human EPO, vertebrate cells capable of producing high levels of EPO using a variety of different expression systems, the complete and accurate sequence of the human EPO gene (Figure 6), and the deduced amino acid sequence of the human EPO protein, just to mention a few. One of skill would have recognized Dr. Lin's patents to have compared favorably with research articles published in peer-reviewed journals. The patent specification summarizes the scope of the inventions disclosed:

> "The present invention relates generally to the manipulation of genetic materials and, more particularly, to recombinant procedures making possible the production of polypeptides possessing part or all of the primary structural conformation and/or one or more of the biological properties of naturally-occurring erythropoietin."[23]

Dr. Lin's patents describe and teach a number of different genetic manipulations useful for making a vertebrate cell produce relatively high levels of human EPO.[24] With Dr. Lin's teachings, the ordinarily skilled molecular biologist would have been fully equipped to practice the inventions broadly, without having to use anything other than routine techniques in molecular biology.

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[23] '933 Col. 1:18-23.

39. Dr. Kingston asserts that Dr. Lin's patents do not describe or teach how to "activate" the "endogenous" EPO gene using homologous recombination.[25] I disagree. As discussed further below, homologous recombination is merely one tool that can be used to make and use Dr. Lin's EPO inventions. Further, Dr. Kingston incorrectly assumes that Dr. Lin's inventions are limited to uses of "exogenous" or "cloned" EPO DNA. But because, as above, Dr. Lin teaches the sequence — the essence — of the "endogenous" human EPO gene, his inventions cannot be so limited.[26]

40. Moreover, Dr. Kingston incorrectly imposes on the claims a distinction between gene "expression" and gene "activation." He implies that gene "activation" is qualitatively distinct from gene "expression," when in fact the two terms are interchangeable in general scientific parlance.[27] "Activating" a gene merely involves turning on its expression.

41. Before Dr. Lin's inventions, it was impossible to genetically manipulate cells to "activate" or express the human EPO gene. Dr. Lin made numerous breakthrough discoveries that proved that one could genetically manipulate the human EPO gene to produce therapeutic human EPO, including:

- Elucidating the sequence of the natural "endogenous" human EPO gene;
- Operatively linking a non-human promoter to the natural "endogenous" human EPO gene to create EPO expression systems;
- Amplifying DNA encoding EPO within vertebrate cells;

[24] *See* '933 Col. 25:30-29:7 (introduction of selectable marker gene and gene amplification); '933 Col. 23:1-24:39 (introduction of expression vector(s) into COS and CHO cells); '933 Col. 36:30-37:51 (various expression systems and approaches).
[25] Kingston ¶ 53.
[26] Additionally, as discussed above, the recited cells of the claims at issue are not limited by how they are prepared but rather by their claimed characteristics. None of those characteristics is dependent upon the location of the human EPO gene to be expressed.
[27] *See, e.g.*, Joyner et al., "Retovirus LTRs Activate Expression of Coding Sequences for the Herpes Simplex Virus Thymidine Kinase" *Gene*, 79:1573-1577 (Mar. 1982) (Exhibit C10).

- Constructing vertebrate cells capable of producing large amounts of human EPO protein having the identical amino acid sequence of human EPO encoded by the "endogenous" EPO gene; and
- Obtaining human EPO in a form and quantity sufficient to characterize its biological activity as being the same as EPO produced from the "endogenous" human EPO gene.

One of ordinary skill would have understood these seminal findings to establish that Dr. Lin taught that the human EPO gene could be "activated" or expressed via genetic manipulation of cells, and that there was nothing intrinsic to the human EPO gene sequence that would prevent its "activation," regardless of its location in the cell.

42. Moreover, as discussed in detail below, defendants' claim to have "activated" the "endogenous" human EPO gene merely confirms both their use of Dr. Lin's inventions and the enduring value of his inventive contributions. Thus, although defendants purport to distinguish their EPO product as having been "gene activated" (*see e.g.*, "GA-EPO"), one of ordinary skill would have appreciated that "gene activated" human EPO was pioneered by Dr. Lin.

Opinion 4

One of ordinary skill in the art would not have accepted Dr. Kingston's various interpretations of Dr. Lin's specification.

43. Throughout his report, Dr. Kingston presents various interpretations of Dr. Lin's specification to support his general assertion that the teachings of Dr. Lin's patents are limited to the uses of exogenous, cloned EPO DNA.[28] As shown below, however, one of ordinary skill in the art would not have accepted Dr. Kingston's various interpretations of Dr. Lin's specification.

44. Dr. Kingston first asserts that because the examples of Dr. Lin's patents involving COS-1 and CHO cells do not in their native state contain any human EPO DNA

sequence, the claimed inventions should be limited to cells containing "exogenous" EPO DNA sequences.[29] The claims at issue, however, do not contain any terms related to any "exogenous" limitation.[30] As discussed above, one of ordinary skill therefore would have understood the claims merely to recite the characteristics of the EPO-producing cells, and not a specific manner in which cells having those characteristics must be created. Moreover, one of skill would have noted that when Dr. Lin wished to limit the scope of his claims to involve only cells containing an "exogenous" DNA sequence, as in the case of '933 claim 6 and '080 claim 7, he expressly did so. Finally, one of skill would have understood the COS-1 and CHO cell examples merely to be exemplary of certain embodiments of the invention and not limiting in nature.

45. Dr. Kingston's next assertion is dependent upon his isolation of the following fragment of a sentence from the specification: ". . .DNA whose high level expression is sought would not have its origins in the genome of the host . . ." Dr. Kingston concludes from this sentence fragment that activation of an endogenous EPO gene in human cells to produce EPO was not part of Dr. Lin's invention.[31] One of ordinary skill, however, would have readily recognized that this sentence fragment has been taken out of context. In its full context, this portion of the specification reads:

> "It will be understood that expression of, *e.g.*, monkey origin DNA in monkey host cells in culture and human host cells in culture, actually constitute instances of 'exogenous' DNA expression inasmuch as the EPO DNA whose high level expression is sought would not have its origins in the genome of the host."[32]

[28] *See, e.g.*, Kingston at 28.
[29] Kingston ¶ 35.
[30] *See e.g.*, '349 claims 1 and 4.
[31] Kingston ¶ 39.
[32] '933 Col. 37:38-43.

By this statement, one of ordinary skill would have easily understood Dr. Lin to be indicating that the high level of EPO expression was not the result of the cell's native state but rather the genetic manipulation made possible by the teachings of the patents.

46. Indeed, defendants used such teachings in order to gain high levels of EPO expression from their cells — cells that have been manipulated according to the teachings of Dr. Lin to contain an "exogenous" DHFR gene for amplification and an "exogenous" promoter sequence for initiating transcription. In any event, one of ordinary skill in the art, unlike Dr. Kingston, would not have derived an "exogenous EPO DNA" limitation from the above-quoted passage because one of skill could have labeled the additional copies of the EPO DNA of the cells exemplified in Example 10 of the specification as being "endogenous" because the additional copies were generated inside the cell.

47. Dr. Kingston next asserts that the specification describes the claimed polypeptides and glycoprotein products as products of the expression of exogenous DNA sequences.[33] But the full context of the passage to which Dr. Kingston refers actually reads:

> "[P]olypeptides . . . uniquely characterized by being the product of procaryotic or eucaryotic host expression (e.g., by bacterial, yeast and mammalian cells in culture) of exogenous DNA sequences obtained by genomic or cDNA cloning or by gene synthesis."[34]

One of ordinary skill would have understood Dr. Lin to be referring to the use of "exogenous DNA sequences" in securing the production of the EPO polypeptides. As discussed above, one of skill could as readily have understood such "exogenous DNA sequences" to include the DHFR gene (necessary to amplify the human EPO DNA),[35] a promoter sequence or another transcription control sequence.

[33] Kingston ¶ 48.
[34] '933 Col. 10:16-20.
[35] '933 Col. 25:46-26:10.

48. Dr. Kingston also asserts that the claimed inventions at issue are limited to the use of a cloned, exogenous EPO DNA based on the following statement from the specification to:

> "Illustrating the present invention are cloned DNA sequences of monkey and human species origins and polypeptide sequences suitably deduced therefrom which represent, respectively, the primary structural conformation of erythropoietins of monkey and human species origins."[36]

One of ordinary skill, however, could readily have understood this statement to reflect that Dr. Lin had disclosed much more than just the use of a "cloned, exogenous" EPO DNA. As discussed above, Dr. Lin's elucidation of the DNA sequence and the primary structural conformation of the "endogenous" human EPO gene gave the world access to, and hence the ability to manipulate, the "endogenous" human EPO gene.

49. Dr. Kingston next asserts that the described plasmid vectors are characterized by "exogenous vector borne [EPO DNA] sequences."[37] But unlike Dr. Kingston, one of ordinary skill would not have altered the language of the specification by reading into it the term "[EPO]." Rather, one of ordinary skill would have read this passage just as it is written:

> "Correspondingly provided by the invention are novel methods for the production of useful polypeptides comprising cultured growth of such transformed or transfected microbial hosts under conditions facilitative of large scale expression of the exogenous, vector-borne DNA sequences and isolation of the desired polypeptides from the growth medium, cellular lysates or cellular membrane fractions."[38]

50. As this language contains no limitation to "**EPO** DNA sequences," one of ordinary skill would have understood the exogenous, vector-borne DNA sequences described and taught in the specification to include the DHFR gene, promoter DNA, and/or transcription

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[36] '933 Col. 10:65-11:2.
[37] Kingston ¶ 48.
[38] '933 Col. 11:8-14.

control sequences alone. And, as described by Dr. Lin, defendants similarly use "exogenous, vector-borne DNA sequences" to express EPO protein in mammalian host cells.

51. Dr. Kingston next asserts that the pharmaceutical compositions taught by Dr. Lin's patents require polypeptides produced from exogenous EPO DNA.[39] Given that the two passages of the specification cited by Dr. Kingston and the claim language at issue do not require that the claimed pharmaceutical compositions be produced from "exogenous EPO DNA," one of skill would have found Dr. Kingston's assertion to be incorrect.

52. For example, '422 claim 1 recites:

> "A pharmaceutical composition comprising a therapeutically effective amount of human erythropoietin and a pharmaceutically acceptable diluent, adjuvant or carrier, wherein said erythropoietin is purified from mammalian cells grown in culture."

One of skill would have found significant the fact that the term "exogenous EPO DNA" does not appear in the claim and that the claim requires only that the EPO protein be "purified from mammalian cells grown in culture." One of skill therefore would have understood that the recited mammalian cells are not limited by the manner in which they are prepared, or the source of the EPO DNA that they contain, or by any requirement that such DNA be exogenous to the cell.

53. The first passage of the specification cited by Dr. Kingston[40] describes a pharmaceutical composition and contains no mention of any exogenous DNA.[41] The second passage of the specification section cited by Dr. Kingston[42] is the same one identified under polypeptide and glycoprotein products, discussed above. Again, the only limitation mentioned

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[39] Kingston ¶ 48.
[40] Kingston ¶ 48.
[41] '933 Col. 12:1-7.
[42] Kingston ¶ 48.

in this section,[43] is the use of "exogenous DNA sequences," which as discussed can include merely the DHFR gene, promoter DNA, and/or other transcription control DNA.

54. Furthermore, Dr. Lin's disclosures and teachings provide numerous examples of applications of the knowledge of the sequence and structure of the human EPO gene and protein that do not require use of a "cloned, exogenous" EPO DNA sequence, including:

- Use of Dr. Lin's inventions to map linked/neighboring DNA sequences within a human or other mammalian chromosome; to identify allelic variants; to locate EPO gene disorders at the DNA level; and as gene markers to identify neighboring genes and their corresponding disorders.[44]

- Manufacturing synthetic EPO polypeptides.[45]

- Preparation of monoclonal antibodies to EPO polypeptides or to recombinant human EPO.[46]

- "Vertebrate (e.g., COS-1 and CHO) cells provided by the present invention comprise the first cells ever available which can be propagated in vitro continuously and which upon growth in culture are capable of producing in the medium of their growth in excess of 100 U (preferably in excess of 500 U and most preferably in excess of 1,000 to 5,000 U) of erythropoietin per 10^6 cells in 48 hours as determined by radioimmunoassay."[47]

- "[P]harmaceutical compositions comprising effective amounts of polypeptide products of the invention together with suitable diluents, adjuvants and/or carriers which allow for provision of erythropoietin therapy, especially in the treatment of anemic disease state and most especially such anemic states as attend chronic renal failure."[48]

- "A preferred method for administration of polypeptide products of the invention is by parenteral (e.g., IV, IM, SC, or IP) routes and the compositions administered would ordinarily include therapeutically effective amounts of product in combination with acceptable diluents, carriers and/or adjuvants."[49]

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[43] '933 Col. 10:9-33.
[44] '933 Col. 12:12-21.
[45] '933 Col. 34:42-67.
[46] '933 Col. 34:42-67.
[47] '933 Col. 10:41-49.
[48] '933 Col. 12:1-7.
[49] '933 Col. 33:50-55.

Finally, because one skilled in the art would have understood that what Dr. Lin had disclosed in the patents was the sequence of the structure of, and the protein produced by, the "endogenous" human EPO gene, they would not have assumed that these examples limit the claimed inventions at issue to embodiments using "cloned, exogenous" EPO DNA.

Opinion 5

Defendants' genetic manipulation of mammalian cells to produce EPO was neither "pioneering" nor "third-wave."

55. I understand that defendants claim to have developed an entirely new way of making EPO and that their method and the resulting EPO product are "pioneering" "third-wave" developments. Defendants' human EPO and methods are neither. Rather, they are merely a logical use of established principles of recombinant DNA engineering long established by others.

56. Defendants' claimed distinctions from Dr. Lin's inventions, *i.e.*, the use of an "endogenous" EPO gene, the use of "targeted" homologous recombination, the use of "human" cells, and the use of a promoter that is not "close" to the EPO gene, are all artifacts of their use of homologous recombination.

57. Homologous recombination techniques alone do not result in the production of EPO. Without Dr. Lin's inventions, the tool of homologous recombination could not have made therapeutically effective human EPO available to the world. Even homologous recombination combined with the prior-art knowledge of human EPO could not have enabled an ordinarily skilled molecular biologist to produce human EPO. Only as part of Dr. Lin's inventions could homologous recombination be used in a process for making EPO. An ordinarily skilled molecular biologist would recognize that homologous recombination, as used

by defendants, is simply one of the ways to practice Dr. Lin's inventions — making human EPO available as a therapeutic.

58. Like PCR (polymerase chain reaction) and automated DNA synthesis, gene targeting and homologous recombination are simply additional tools for manipulating DNA to create recombinant DNA molecules and recombinant cells useful in producing recombinant proteins. Defendants' use of an ancillary technique such as gene targeting in their practice of Dr. Lin's inventions does not mean they are not practicing Dr. Lin's patents. If defendants were permitted to use gene targeting or homologous recombination to avoid literally infringing Dr. Lin's patents, such a result would have broad negative implications for the field of biotechnology. If defendants were able to circumvent Dr. Lin's EPO patents on cells, therapeutic compositions, and methods of producing EPO by merely incorporating an ancillary, more recent technique, the consequence would be that parties could avoid infringement by simply adding a new step or aspect beyond the claim limitations to their methods or product.

59. Furthermore, defendants' method is largely derivative. For example, defendants did not invent gene targeting using homologous recombination. Rather, others developed such tools well before defendants' "GA-EPO" project began. As another example, defendants did not invent the method of using homologous recombination to insert a viral promoter upstream of a chromosomal human gene in a human cell to achieve high-level expression of the gene. Others researchers developed such methods well before defendants. U.S. Patent No. 5,272,071, entitled "Method for the Modification of the Expression Characteristics of an Endogenous Gene of a Given Cell Line"[50] (PCT filing date of Dec. 21, 1990) contains claims to using homologous recombination to insert a viral promoter upstream of a chromosomal gene in a human cell to

[50] Exhibit E.

achieve expression of the gene.[51] This patent illustrated to those of ordinary skill how little defendants' methods differ from what others had already invented.

60. Even defendants' use of an unpaired splice donor site to create an artificial intron of more than 1 kilobase pairs in length was not novel. Introduction of an artificial splice donor site to remove an unnatural intervening sequence of greater than 1 kilobase in length in a protein expression vector was known and used by others well before defendants filed their first application describing an unpaired splice donor site in 1991.[52]

61. Defendants claim to have developed a "third-wave," "pioneering" invention distinct from a "second-wave" of recombinant DNA inventions. In effect, they argue that their use of homologous recombination to produce proteins in mammalian cells in culture renders the entire field of expressing proteins from cloned DNA sequences obsolete. I disagree. One of ordinary skill would have found no significant difference between Dr. Lin's claimed human EPO and defendants' "GA-EPO" because both are expressed in mammalian cells from the same DNA sequence encoding human EPO. Likewise, Dr. Lin's patented cells and defendants' cells are identical for purposes of human EPO production — both contain multiple copies of the "endogenous" human EPO gene and both express the specified high levels of human EPO. Moreover, defendants' cells, like specific embodiments of Dr. Lin's claimed cells, were genetically engineered to contain recombinant human EPO genes operatively linked to exogenous non-human promoter sequences, which have been amplified through MTX selection.

[51] Claim 1 of U.S. Pat. No. 5,272,071 provides: A method of activating a predetermined normally transcriptionally silent gene within the genome of a cell line so as to enable said cell line to express the gene product of said gene, comprising inserting a DNA construct into said genome by homologous recombination, said DNA construct comprising a DNA regulatory segment capable of stimulating expression of said gene when operatively linked thereto and a DNA targeting segment homologous to a region of said genome within or proximal to said gene, wherein said construct is inserted such that said regulatory segment is operatively linked to said gene of interest.

For these reasons and because defendants' cell lines are recombinant cells expressing recombinant proteins and their derivative methods merely incorporate the tool of homologous recombination, I do not consider defendant's methods or product to be "third-wave" or "pioneering."

62. Those of ordinary skill in the field would not find any scientific benefit to defendants' minor variation on Dr. Lin's inventions involving operatively linking a non-human promoter to DNA encoding human EPO. First, defendants' use of gene targeting is a minor variation that does not involve a critical limitation of any of the claims at issue. Similarly, the physical location where Dr. Lin's recombinant DNA vector inserted into the cellular chromosomal DNA has no meaningful scientific effect on EPO expression in the claimed cells versus in defendants' cells for producing EPO. Whether the DNA vector is inserted at the human EPO gene's native location or is randomly integrated throughout the chromosome, it has no effect on the selected transformed vertebrate cell's production of human EPO or the nature of the resulting human EPO. For these reasons, I conclude that defendants' distinctions between "exogenous" and "endogenous," transcription control sequences "immediately adjacent to" or at a substantial distance from DNA encoding human EPO, and random or targeted integration of "exogenous" DNA sequences, are all meaningless. Such attempted distinctions have no apparent effect on the resulting human EPO.

Opinion 6

Defendants could not have achieved expression of human EPO in human cells without using Dr. Lin's invention.

63. To develop their cell line for producing human EPO, defendants were critically dependent on Amgen's human genomic EPO DNA sequence, the protein sequence, the

[52] *See, e.g.*, D. Kaetzel et. al., *PNAS USA*, 82:7280-7283 (Nov. 1985) at 7281 (Exhibit C14); and Treco

vertebrate cells having amplified DNA encoding EPO and/or having non-human transcription control sequences linked to DNA encoding EPO, and many other Amgen inventions.

64. Based on my review of TKT's U.S. Patent No. 5,641,670 (the '670 Patent)[53] and defendants' IND,[54] it is plain that defendants relied heavily on Dr. Lin's teachings. Following is a listing of Dr. Lin's information that defendants used and how they used it:

- Defendants used Dr. Lin's methods for amplifying a human EPO gene in cells to produce large quantities of human EPO.

- Defendants used Dr. Lin's vertebrate cell inventions, *e.g.*, they created a recombinant mammalian cell containing a mouse DHFR gene, a viral promoter, and a DNA sequence from the "endogenous" EPO locus, to obtain high expression levels of human EPO.

- Defendants introduced a recombinant DNA into mammalian cells in culture under conditions permitting integration of the recombinant DNA into the cell's chromosome, producing a recombinant EPO locus and recombinant EPO.

- Defendants synthesized probes containing part of the Figure 6 sequence to isolate and characterize human genomic DNA clones containing the EPO gene locus. These isolated clones were used to create their recombinant DNA targeting construct.[55]

- Defendants used Dr. Lin's human EPO gene sequence to design their targeting construct to recombine into the chromosome upstream of the EPO structural gene.

- Defendants used the length, sequence and structure of the EPO signal peptide and the sequence and structure of the human EPO first exon/first intron/second exon regions to create an artificial first exon to replace the natural human genomic EPO first exon.

- Defendants used a sequence from Dr. Lin's Figure 6 sequence, *i.e.*, the 5' splice donor site from the EPO first exon/first intron junction in

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U.S. Patent 5,641,670 (Exhibit C12).
[53] *See* Exhibit D, at 26:62-27:45.
[54] Exhibit C13, [redacted]
[55] *See* Example 1.f of '670 patent, col. 26:61-66. (TKT 0067657-658) (Exhibit D).

their targeting construct, which splice donor site defendants acknowledge is critical to their homologous recombination method.[56]

65. This extensive listing demonstrates that defendants could not have expressed human EPO in recombinant cells without using Dr. Lin's inventions. Moreover, it illustrates the striking similarities between defendant's methods and Dr. Lin's.

66. The fact that Dr. Lin's recombinant DNA integrated randomly into the chromosome and defendants' recombinant DNA integrated at a specific site in the chromosome is not scientifically significant. Both recombinant DNAs are amplified upon MTX selection, resulting in amplified copies of the "endogenous" human EPO gene and increased expression of EPO in selected transformed cells. And, Dr. Lin proved that amplification of DNA encoding EPO would lead to increased expression of biologically active EPO.

67. There is no discernible scientific benefit to defendants' minor variations on Dr. Lin's inventions. Whether one uses human cells or other mammalian cells, "endogenous" or "exogenous" EPO DNA sequences, targeted or random integration of DNA sequences into the cells' chromosome, or a promoter immediately adjacent to or at a distance from the EPO coding sequence, I am not aware of any advantages or distinguishing features of the resulting human EPO for pharmaceutical use.

VI. COMPENSATION

68. I have been retained by counsel for Amgen as a consultant in connection with this action since October 26, 1999. Under my consulting agreement, I am paid at my usual hourly rate of $250.00. I have received no additional compensation for my work on this action.

[56] *See* Exhibit D, '670 patent, TKT 67662

VII. PREVIOUS TESTIMONY

69. I have not testified as an expert at trial or by deposition within the preceding four years.

VII. TRIAL EXHIBITS

70. I may rely on visual aids that demonstrate the bases of my opinion. Examples of these visual aids and demonstrative exhibits may include: claim charts, patent drawings, excerpts from patent specifications, file histories, interrogatory responses, deposition testimony, deposition exhibits, charts, diagrams, videos, and animated or computer-generated video presentations describing the technology relevant to the patents-in-suit and the prior art.

Dated: January 24, 2000 in La Jolla, California.

Richard D. Kolodner

RICHARD D. KOLODNER, PH.D.